REMARKS

In this Amendment, claim 27 is amended and claim 33 is new. Thus, after entry of this Amendment, claims 27, 29, 30 and 33 are pending in the application.

Claim 27 has been amended to remove the TS2/18 CD2 antibody limitation, which is now recited in new claim 33.

Claim 27 has also been amended to remove the term "activation" from the preamble, as supported by page 1 of the specification. This amendment is intended only for clarity, and is not intended to be narrowing.

Claim 27 has also been amended to recite that the cultured immunosuppressive cells "have an ability to suppress hypersensitivity of an immune system which causes autoimmune disease." This amendment is supported by page 3, lines 3 to 5 of the specification.

No new matter has been added.

Applicants respectfully request entry of the Amendment.

Claim Rejections

(A) At page 2 of the Office Action, claims 27, 29, and 30 are rejected under 35 USC § 103(a) as obvious over Skibbens in view of Schwarz, Chavin (1994), Chavin (1993), and Jones. Specifically, the Examiner contends that the references teach the following.

The Examiner contends that Skibbens teaches a culture device for the culturing of immunosuppressive cells, wherein the culture device is coated with an anti-CD3 antibody

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(OKT3). The Examiner admits that Skibbens does not teach a device further coated with the $F(ab)_2$ fragment of the anti-CD2 antibody TS2/18 produced by the hybridoma HB-195.

The Examiner contends that Schwarz teaches culturing T cells with the anti-CD2 antibody TS2/18 and that such results in inhibitory effects on T cell activation. The Examiner contends that Schwartz also teaches that the epitope recognized by TS2/18 is a candidate for CD2-directed immunosuppression.

The Examiner contends that Chavin (1994) teaches that anti-CD2 antibodies can be used to generate Th2 suppressor cells and that various anti-CD2 antibodies are interchangeable.

The Examiner contends that Chavin (1993) teaches that anti-CD2 and anti-CD3 antibodies synergize in an immunosuppression context.

Finally, the Examiner contends that Jones teaches that whole antibodies and $F(ab)_2$ fragments are interchangeable in the coating of devices for the incubation of lymphocytes. The Examiner further contends that Jones teaches that $F(ab)_2$ fragments may be preferable when, for example, the reduction of a background signal is desirable.

The Examiner concludes that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to coat a culture device with an anti-CD3 antibody, as taught by Skibbens, and the anti-CD2 antibody TS2/18 as taught by Schwarz, employing either the whole antibody or the $F(ab)_2$ fragment in view of the teachings of Jones. The Examiner believes that one of ordinary skill in the art would have been motivated to coat the device with both an anti-CD2 and anti-CD3 antibody given the teachings of the Chavin references.

With regard to Applicants' Remarks filed May 21, 2004, that the invention has unexpectedly superior properties, the Examiner believes that the properties of the present invention cannot be considered unexpected given the teachings of Chavin (1993) that anti-CD2 and anti-CD3 antibodies synergize in an immunosuppression context.

(B) At page 4 of the Office Action, claims 27, 29, and 30 are rejected under 35 U.S.C. § 103(a) as obvious over EP'380 in view of Schwarz, Chavin (1994), Chavin (1993), and Jones (1992) as applied above. Specifically, the Examiner contends that EP'380 teaches a culture device coated with an anti-CD3 antibody and an anti-CD2 antibody that may include enzymatically cleaved antibody fragments. The Examiner admits that EP'380 does not teach the specific anti-CD2 antibody TS2/18 produced by the hybridoma HB-195 nor the use of a $F(ab)_2$ fragment, but the Examiner believes that the secondary references as applied above are sufficient in this regard.

Applicants' Response to Claim Rejections

The present invention relates to a culture device for inducing immunosuppressive cells. The device comprises a container coated with the $F(ab)_2$ fragment of at least one anti-CD2 antibody and at least one anti-CD3 antibody, wherein the immunosuppressive cells have an ability to suppress hypersensitivity of an immune system which causes autoimmune disease.

Applicants request reconsideration and withdrawal of the present 103 rejections, since the references alone or in combination do not suggest the present invention, and in addition, the present invention provides results that are unexpected over the cited references.

With regard to the rejection over Skibbens in view of the secondary references, Applicants submit that none of the references teaches a device for culturing immunosuppressive cells with an ability to suppress hypersensitivity of an immune system which causes autoimmune disease. As such, the Examiner has not established a *prima facie* case of obviousness.

The Examiner states at page 2 of the Office Action that "[t]he '799 Patent (Skibbens) teaches a culture device for the culturing of immunosuppressive (suppressor) cells...." However, Applicants respectfully submit that no suppressor cell is disclosed in Skibbens that can suppress hypersensitivity of an immune system which causes autoimmune disease. Skibbens states at column 33, lines 32-33, "[s]uppressor activity was determined by the ability of a γδ+ cell culture supernatant to inhibit the proliferative response of PBLs to OKT3 or PHA."

The supernatant described in Skibbens does not contain cells, and thus, the primary reference relied upon by the Examiner does not teach or suggest a culture device for inducing immunosuppressive cells that themselves have an ability to suppress hypersensitivity of an immune system which causes autoimmune disease, as recited in the present claims. The present invention is directed to a device for culturing immunosuppressive cells, especially immunosuppressive T cell as described at, for example, page 2, lines 24-25 of the specification. The present invention uses immunosuppressive cells *per se* in order to suppress T cell activation.

In addition, Applicants submit that while the supernatant of Skibbens may suppress various T cells of no particular nature that are contained in normal peripheral blood lymphocytes, the immunosuppressive cells induced by the present invention suppress particular T cells, i.e. T cells activated with PPD, which is regarded as a causative agent of autoimmune diseases (see

Test Example 1). Therefore, the immunosuppressive cells of the invention will be suitable for treatment of autoimmune disease, as recited in the instant claims.

Regarding the rejection over EP'380 as the primary reference, the device disclosed by EP'380 for inducing tumor-lysing cells contains anti-leukocyte antibodies including OKT3. In EP'380, tumor-lysing cells are cells that have the function of lysing tumor cells (page 3, lines 31 to 32), such as cytotoxic T lymphocytes, natural killer cells and monocytes (page 1, lines 4 and 5). Further, in EP'380, the induction of tumor-lysing cells refers to activation of killer activity (page 3, lines 41 to 42). That is, the device of EP'380 induces cytotoxic cells, the activity of which the present invention aims to suppress using immunosuppressive cells. Therefore, the target of induction of EP'380 clearly differs from that of the present invention, and thus cannot be combined with any reference for the purpose of suppressing T-cells, as such changes the fundamental nature of the device of EP'380.

Further, regarding the combination of the secondary references, Applicants submit the following.

The Examiner states that Schwarz teaches the culture of T cells with the anti-CD2 TS2/18 antibody, and that this culture results in inhibitory effects on T cell activation, i.e., anti-CD2 TS2/18 antibody inactivates the T cells. Schwartz also states that "[o]ne plausible explanation for our finding would be that TS2/18 interferes with the association of CD2 with other surface or intracellular proteins and, in this way, interferes with optimal signal transduction through CD2" (page 5818, right column, line 11-14). Thus, those skilled in the art who wish to activate T cells

and to thereby induce immunosuppressive cells would be discouraged to use TS2/18, as recited by claim 33.

On the other hand, the immunosuppressive cell of the present invention are made through activation of T cells by contacting the T cells with $F(ab)_2$ fragment of at least one anti-CD2 antibody and at least one anti-CD3 antibody. These activated T cells acquire an ability to suppress other T cells which will cause immunosuppressive diseases. Please see the specification, page 13, line 20-24, stating: As explained in the following TEST EXAMPLE 1 and 2, in vitro, cells probably having immunosuppressive activity are mixed with human peripheral blood lymphocytes (hereinafter also referred to as PBL) that is newly obtained from the same person. Schwarz neither discloses nor suggests such contact.

Regarding Chavin (1994), the Examiner states "Chavin et al (1994) teaches that anti-CD2 antibodies can be used to generate Th2 suppressor cells and that various anti-CD2 antibodies are interchangeable."

Applicants submit that Chavin (1994) induces immunosuppressive cells *in vivo*, and does not suggest that such cells should be or could be induced *in vitro*. It is recognized by those of skill in the art that the *in vivo* and *in vitro* events are quite different. For example, Chavin states "the administration in vivo of equimolar amounts of $F(ab')_2$ anti-CD2 only partially inhibited CTL, suggesting that Fc-related signaling might be important" (page 3731, Results, first paragraph). They conclude that "the precise nature of the Fc-related immunosuppressive events is uncertain; however, they may facilitate intercellular interaction and induction of regulatory cell subsets (page 3736, right column, line 10-13).

However, to the contrary, immunosuppressive cells are effectively induced *in vitro* when the Fc fragment is removed as shown in Fig. 6 of the present application. Therefore, Chavin does not suggest that the $F(ab)_2$ fragment of at least one anti-CD2 antibody and at least one anti-CD3 antibody may be used to induce immunosuppression *in vitro*, and as such, Chavin (1994) is improperly combined with the primary references, which allegedly teach *in vitro* culture devices.

Further, regarding Chavin (1993), the Examiner states that anti-CD2 and anti-CD3 antibodies synergize in an immunosuppression context. However, the immunosuppression of Chavin (1993) also involves *in vivo* induction, which does not suggest that anti-CD2 and anti-CD3 antibodies synergize in an immunosuppression context when coated *in vitro*. Thus, Chavin (1993) is improperly combined with the primary references allegedly teaching culture devices.

Regarding Jones, Jones describes that "such heterophil antibody interference can usually be eliminated by the inclusion of large amounts of absorptive, non-immune serum in the diluents or the use of antibodies that have had their Fc portions enzymically removed (page 238, bottom of left column). In other words, Jones suggests that, due to the lack of the Fc fragments, it is possible to eliminate Fc-mediated non-specific antibody binding, which teaching is irrelevant to the present invention, and as such, does not suggest to one of skill in the art to prepare a culture device for inducing immunosuppression *in vitro* using the $F(ab)_2$ fragment of a anti-CD2 antibody.

Consequently, the references cited by the Examiner are improperly combined, and as such, the Examiner has not made a *prima facie* case of obviousness.

Regarding the Examiner's statement that "the properties of the instant invention cannot be considered to be unexpected given the teachings of Chavin et al. (1993) that anti-CD2 and anti-CD3 antibodies have been shown to synergize in an immunosuppression context," Applicants respond as follows.

In Chavin (1993), a synergistic immunosuppressive effect was observed when administration of an anti-CD3 antibody was delayed as to the anti-CD2 antibody, and the immunosuppressive effect was only additive when identical doses of monoclonal antibodies were administered concurrently (page 906, left column, lines 37 to 41, and page 903, right column, lines 20 to 28). In fact, none of the cited reference, including Chavin (1993), describe nor suggest that the combination of $F(ab)_2$ fragment of anti-CD2 antibody and anti-CD3 antibody in a culture device provides a synergistic immunosuppressive effect.

The present invention relates to a culture device coated with both $F(ab)_2$ fragment of anti-CD2 antibody and antiCD3 antibody. According to TEST EXAMPLE 1 of the present specification, the effect of the present invention is that activity of T cells is suppressed 78%, in contrast to when $F(ab)_2$ fragment of anti-CD2 antibody alone and anti-CD3 antibody alone are used, whereby the activity is suppressed 20% and 50% respectively. Hereinafter, it is described that the result of 78% suppression is an unexpected and synergistically increased effect.

In TEST EXAMPLE 1 of the present specification, the combination of $F(ab)_2$ fragment of anti-CD2 antibody (10 ug/ml) and anti-CD3 antibody (10 ug/ml) is used, and a total of 20 ug/ml is used. On the other hand, in the case of $F(ab)_2$ fragment of anti-CD2 antibody alone and anti-CD3 antibody alone, the amount used is 10 ug/ml respectively, and is not 20 ug/ml. However,

this is because it is generally considered in the art that the maximal effect is reached with 10 ug/ml of an antibody, and the effect is not increased after 10 ug/ml. In addition, this is clear from the description of Buysmann et al (1996) which is attached hereto, stating, "both the increase in NKI-L16 and CDllb expression were dose dependent, reaching a maximal effect with 10 ug/ml for OKT3" (page 407, right column, lines 19 to 21). Therefore, the amount used is 10 ug/ml respectively, in the case of $F(ab)_2$ fragment of anti-CD2 antibody alone and anti-CD3 antibody alone, because the same result is obtained even if 20 ug/ml is used.

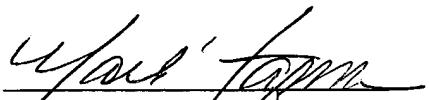
Further, since the effect is not enhanced past l0 ug/ml of an antibody, those skilled in the art would consider that even if $F(ab)_2$ fragment of anti-CD2 antibody (l0 ug/ml), which has 20% suppression effect, is combined with anti-CD3 antibody (10 ug/ml), which has 50% suppression effect, the effect of the combination is at most 50% suppression. Thus, the result of 78% suppression of the present invention, by combining and concurrently using $F(ab)_2$ fragment of anti-CD2 antibody and anti-CD3 antibody, is an unexpected and synergistically increased effect.

Therefore, the present invention provides results that are unexpected over the combined teachings of Skibbens, Schwarz, Chavin (1994), Chavin (1993) and Jones, and EP'380.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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Activation and Increased Expression of Adhesion Molecules on Peripheral Blood Lymphocytes Is a Mechanism for the Immediate Lymphocytopenia After Administration of OKT3

By Saskia Buysmann, Frederike J. Bemelman, Peter Th.A. Schellekens, Yvette van Kooyk, Carl G. Figdor, and Ineke J.M. ten Berge

We investigated the mechanism by which antihuman CD3 monoclonal antibodies of the isotypes IgG2a (eg, OKT3) and IgA (eg, IXA) can induce the rapid disappearance of virtually all circulating T lymphocytes. We hypothesize that upregulation of adhesion molecules on the lymphocyte membrane contributes to this effect. However, this hypothesis is difficult to test, because of the inherent lymphocytopenia and/or shifts in lymphocyte populations between intra and extravascular compartments. Therefore, studies in vitro were performed, as well. Analysis of peripheral blood lymphocytes isolated at several times after addition of OKT3 or IXA to whole blood of healthy individuals showed an immediate increase in the proportion of T cells expressing NKI-L16, an activation epitope on CD11a/CD18. Likewise, an increase in CD11b/CD18 expression occurred. In parallel experiments, a transiently increased adhesion of T cells to endothelial cell monolayers was observed. This adhesion could be completely blocked by anti-CD18 or anti-CD11a monoclonal antibodies and only partly by an anti-CD11b antibody. Our data indicate that upregulation of activation epitopes of CD11a/CD18, as well as increased expression of CD11b/CD18 on T lymphocytes, may result in increased adhesion of these cells to intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 on vascular endothelium. This phenomenon may, at least, partly explain the rapidly occurring peripheral lymphocytopenia observed in vivo.

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IT IS well-established that OKT3, a murine IgG2a monoclonal antibody directed against the CD3 molecule on human T lymphocytes, is a potent immunosuppressive agent in prophylaxis and treatment of acute allograft rejection.[1-3] It is generally assumed that the concomitant immediate and profound peripheral blood lymphocytopenia[4] is caused by sequestration of OKT3-sensitized T lymphocytes, following binding to Fc- and complement receptors.[5-7] However, this cannot be the sole explanation, in particular for the initial phase of T lymphocytopenia. Indeed, previous studies with murine isotype switch variant CD3 monoclonal antibodies in chimpanzees, as well as humans, have shown that the IgG2a, as well as IgA isotypes, can induce an immediate, although transient, lymphocytopenia.[8,9] Because neither chimpanzees nor humans express Fc receptors for murine IgA and monomeric IgA is generally assumed to have poor complement-activating capacity,[9] the rapidly occurring lymphocytopenia induced by the anti-CD3 monoclonal antibodies must have other causes. We hypothesize that binding to vascular endothelium via adhesion molecules is instrumental in the rapidly occurring peripheral lymphocytopenia.

In vivo, adhesion of peripheral blood lymphocytes to endothelium is a multiple-stage process, starting with a low-avidity contact via CD62L (L-selectin) and resulting in the rolling of lymphocytes along the endothelial cell layer, thus facilitating the binding of activated integrins such as CD11a/CD18 (lymphocyte function associated antigen-1, LFA-1), CD11b/CD18 (Mac-1), and CD49d/CD29 (very late antigen-4, VLA-4) to their respective ligands: intercellular adhesion molecule-1 and -2 (ICAM-1 and ICAM-2) and vascular cell adhesion molecule-1 (VCAM-1). These interactions result in a high-avidity binding of lymphocytes to endothelium.[10] Likewise, incubation of T lymphocytes with anti-CD3 monoclonal antibodies in vitro has been shown to result in a transient high-avidity state of the CD11a/CD18 complex and an increased adhesion to ICAM-1–coated surfaces. This effect was maximal after 10 minutes and subsided within 30 minutes to 2 hours.[11] To test our hypothesis, we investigated in vivo, as well as in vitro, whether OKT3 induces not only an increase in the avidity of the CD11a/CD18 complex, but also of other adhesion molecules on T lymphocytes. Thus, CD2 positive and CD3 positive peripheral blood lymphocytes were studied for their expression of CD62L, CD11a/CD18, NKI-L16 (a Ca^{2+}-dependent epitope on the CD11a/CD18 complex, which is only expressed on activation),[12,13] CD11b/CD18, and CD49d/CD29. Time-course studies were performed just before and at frequent time intervals after administration of OKT3 to patients with acute kidney allograft rejection. Unfortunately, we were unable to perform similar studies with IgG2a and IgA isotype switch variants of CD3 monoclonal antibodies, because such variant antibodies were not available for clinical use. A control group consisted of patients with acute kidney allograft rejection who were treated with high-dose methylprednisolone only.

Moreover, studies in vitro were performed in which peripheral blood lymphocytes were incubated at 37°C in the presence of OKT3 or the IgA isotype switch variant anti-CD3 antibody, after which the expression of adhesion molecules was studied. Furthermore, we showed that an increased expression of adhesion molecules will result in an enhanced adhesion of T lymphocytes to endothelial cell monolayers. Finally, blocking studies were performed to identify the adhesion molecule(s) that are responsible for this increased adhesion.

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MATERIALS AND METHODS

Methods

The study was approved by the institutional research and ethical committees of the Academic Medical Center of the University of Amsterdam. Written informed consent was obtained from all patients.

In Vivo Studies

Patients. Ten renal transplant recipients (three men, seven women, median age 31 years, range, 20 to 61 years) were studied. These patients were treated for 10 consecutive days with 5 mg/day OKT3 (Ortho Diagnostic Systems, Raritan, NJ) because of acute kidney allograft rejection, diagnosed on the basis of clinical manifestations and confirmed by core biopsy. As recommended by the manufacturer, before OKT3 administration on day 1, a single dose of 500 mg methylprednisolone was given. The control group consisted of 10 renal transplant recipients (four men, six women, median age 31 years, range, 21 to 54 years) who were treated for acute rejection with methylprednisolone 500 mg/day for 6 consecutive days. Basic immunosuppressive therapy in both groups consisted of prednisolone (10 mg/day) and cyclosporin (dosage adjusted so as to result in whole-blood trough levels of 150 to 200 μg/L).

Materials and methods. Peripheral blood was collected before the administration of 500 mg methylprednisolone and at 3, 10, 15, 30, 60, 120 minutes, 4.5, and 24 hours after administration of either 5 mL (ie, 5 mg) OKT3 or 5 mL 0.9% saline (control group). At each time point, total leukocyte and differential counts were determined by flow cytometry (Technicon H1 System; Bayer/Technicon, Tarrytown, NY) on blood anticoagulated with 0.38 mmol/L EDTA. Peripheral blood mononuclear cells were isolated by centrifugation of heparinized blood on Ficoll-Paque (density 1.076; Pharmacia, Uppsala, Sweden). Contaminating erythrocytes were lysed by incubation with ammoniumchloride (155 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$, 0.1 mmol/L EDTA, pH 7.4) for 10 minutes on ice, followed by two washings of the mononuclear cells in PBS-A (ie phosphate buffered saline (PBS) containing 0.5% wt/vol bovine serum albumin (Boseral DEM, Organon Teknika B.V., Boxtel, Holland) and 0.01% wt/vol sodium azide).

Immunofluorescence. Peripheral blood mononuclear cells were incubated for 30 minutes at 0°C in PBS-A with appropriate dilutions of the fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies CD11a (Dako, Glostrup, Denmark, clone MHM24, IgG1k), NKI-L16[12,13] and CD16 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands; clone CLB-149, IgG2a), and with the phycoerythrin-labeled monoclonal antibody CD11b (Dako; clone 2LPM19c, IgG1k). For determination of CD62L and CD49d/CD29 expression, an indirect staining procedure was used consisting of primary incubation of the mononuclear cells with appropriate dilutions of the monoclonal antibodies LECAM-1 (directed against CD62L, Becton Dickinson, San Jose, CA; clone SK11, IgG2a, biotinylated) or CD49d (directed against VLA-α4 chain, Immunotech, Marseille, France; clone HP2.1, IgG1), followed by incubation with phycoerythrin-labeled streptavidin (Becton Dickinson) or FITC-labeled goat antimouse IgG1 (Southern Biotechnology, Birmingham, AL), respectively. All samples were two-color stained with a phycoerythrin- or FITC-labeled anti-CD2 monoclonal antibody (Dako; clone MT910, IgG1k or Becton Dickinson, Leu-5b; clone S5.2, IgG2a, respectively) and with a phycoerythrin- or FITC-labeled anti-CD3 monoclonal antibody (both from Becton Dickinson, Leu-4; clone SK7, IgG1). Determination of the absolute number of CD3 positive cells was performed in two ways: (1) direct labeling with a phycoerythrin- or FITC-labeled anti-CD3 monoclonal antibody (Becton Dickinson, Leu-4; clone SK7, IgG1) and (2) with FITC labeled $F(ab')_2$ fragments of goat antimouse Ig (GAM $F(ab')_2$, obtained from the CLB. Analysis of cell suspensions was performed on a FACScan (Becton Dickinson). Lymphocytes in the mononuclear cell population were gated on forward and side scatter.

In Vitro Studies

Immunofluorescence. A total of 55 mL heparinized peripheral blood was taken from five healthy individuals and kept in a 37°C waterbath. A 5-mL sample was set apart and the remaining 50 mL blood was divided into four separate portions, to which OKT3, IXA, an irrelevant murine monoclonal antibody of the IgG2a isotype (F23-49, directed against *Mycobacterium* tuberculosis[14]) or an irrelevant murine monoclonal antibody of the IgA isotype (clone TEPC15, mouse IgAκ myeloma protein; Sigma, St Louis, MO) was added, each at a final concentration of 1 μg/mL, which is comparable to serum levels after administration of 5 mg OKT3 in vivo.[15] At 3, 30, and 60 minutes after addition of the monoclonal antibody, 3 mL samples were taken. Peripheral blood mononuclear cells were separated by Ficoll-Paque density gradient centrifugation and expression of the same adhesion molecules as analyzed in the in vivo studies was measured on a FACScan as described above. At each time point, total leukocyte and differential counts were determined by flow cytometry (Technicon H1 System).

Functional Adhesion Studies

From eight healthy individuals, 40 mL heparinized peripheral blood was taken. Mononuclear cells were isolated by centrifugation on Ficoll-Paque, followed by three washings in Tris-buffered Earle's Balanced Salt Solution containing 5% fetal calf serum. Next, $CD3^+$ T cells were obtained by a negative selection procedure: removal of B lymphocytes, monocytes, and natural killer cells by incubation with murine monoclonal antibodies anti-CD19 (CLB, clone 11G1), anti-CD14 (CLB, clone 8G3), and anti-CD16 (CLB, gran-1) followed by incubation with sheep antimouse IgG coated Dynabeads (Dynal A.S, N-0212, Oslo, Norway). This procedure resulted in >95% pure T-cell suspensions as confirmed by fluorescence-activated cell sorter (FACS) analysis after staining the cells with a mixture of FITC-labeled CD3- and phycoerythrin-labeled CD16 and CD56 monoclonal antibodies (NK-Simulset, Becton Dickinson). The CD3-positive cells (2×10^6 cells/mL) were incubated on ice for 30 minutes with OKT3, IXA, the irrelevant IgG2a monoclonal antibody F23-49, or the irrelevant IgA monoclonal antibody TEPC15, each at a final concentration of 1 μg/mL, followed by two washings at 4°C with Iscove's modified Dulbecco's medium containing 5% fetal calf serum. Next, 2 μg/mL goat antimouse Ig (GM17D, CLB) was added to cross-link the T-cell receptor/CD3 complexes, immediately followed by 3 minutes 10g centrifugation at 37°C.[11] Part of the cell suspension was then added to a cell monolayer consisting of an immortalized endothelial cell line derived from human umbilical vein endothelial cells,[16] cultured in 96-well plates (Nunclon Micro Well F96, Nunc A.S, Roskilde, Denmark) on 1% gelatin (Sigma, G1890) in PBS. The remaining cell suspension was placed in a shaking waterbath at 37°C and after 30 and 60 minutes of incubation, samples were added to the endothelial cell monolayers. The 96-well plates were incubated for 15 minutes at 37°C in 5% CO_2, followed by removal of the nonadherent fraction by washing three times with 200 μL 37°C Earle's Balanced Salt Solution containing 5% fetal calf serum. The adherent fraction was stained with Diff Quick (a 3-step staining procedure comprising: (1) fixation in methanol with fast green 0.002 g/L, (2) eosin G in phosphate buffer pH 6.6, and (3) thiazine dye in phosphate buffer pH 6.6 (Baxter Dade A.G, Düdingen, Switzerland). All experiments were performed in triplicate. As a positive control, the cells were preincubated with phorbol myristate acetate (50 ng/mL, Sigma) instead of a CD3 monoclonal

antibody. Blocking studies were performed with monoclonal antibodies directed against the common β_2 integrin chain (CD18): TS 1/18,[17] against CD11a (NKI-L7),[18] and against CD11b (Bear-1[19]), added to the endothelial cell monolayer together with the T cells. The final antibody concentration was 10 μg/mL, which in previous studies was shown to result in a maximal inhibition of adhesion. The adhesion was scored blindly at 200 × magnification by three investigators independently, in a semiquantitative way: 0 = no T cells, 1 = <50 T cells/field, 2 = 50 to 100 T cells/field, 3 = 100 to 250 T cells/field, 4 = 250 to 350 T cells/field, 5 = >350 T cells/field.

Because it is difficult to estimate the extent to which cross-linking occurs in vivo, since we do not know which part of the administered amount of CD3 monoclonal antibody actually binds to the T cells and which part remains unbound, both the whole blood incubation and adhesion studies were performed with concentrations of OKT3 or 1XA ranging from 0.01 to 10 μg/mL. To exclude the possibility that the expression and functional activity of adhesion molecules was influenced by any antibody not directed to the CD3/T-cell receptor complex, whole blood incubations and adhesion studies were also performed with murine IgG2a monoclonal antibodies directed against either the CD4 molecule (ie, CLB-T4), or a major histocompatibility complex (MHC) class-I antigen (W6/32), instead of the CD3 monoclonal antibody. Both the CD4 and the MHC class-I monoclonal antibody were tested at concentrations of 0.1, 1, and 10 μg/mL.

Calculations and Statistics

Absolute number of double-positive cells. This was determined by the percentage of double-positive cells (calculated by means of PCLYSYS software from Becton Dickinson) multiplied by the absolute number of lymphocytes at the same time point. All values are expressed as mean ± standard error of mean (SEM).

Mean fluorescence intensity (MFI). At all time points the MFI was calculated by means of PCLYSYS software. A total of 100% represents the MFI values obtained from the blood samples before the start of treatment. The values obtained several times after administration of OKT3 are represented as a percentage of the pretreatment value. All values are expressed as mean ± SEM.

Statistical analysis. Differences within groups were tested by Wilcoxon Test for Matched pairs. A probability (P) value < .05 was considered to indicate a significant difference.

RESULTS

In Vivo Studies

Effect of OKT3 administration on peripheral T lymphocytes. As seen in Fig 1, treatment with 5 mg OKT3 caused within 3 minutes in all of the 10 patients studied a sharp decline in the number of CD3⁺ peripheral blood lymphocytes (from 1.24 ± 0.15 × 10^9/L to 0.07 ± 0.01 × 10^9/L, P < .05). From 3 minutes on, the number of goat antimouse F(ab')$_2$ positive cells decreased in parallel, while the number of CD3⁺ cells remained very low during the whole study period. Likewise, the absolute number of CD2⁺ peripheral blood lymphocytes showed within 3 minutes a rapid decrease from 1.40 ± 0.16 × 10^9/L to 0.54 ± 0.06 × 10^9/L (P < .05). Afterwards, the decrease in CD2⁺ lymphocytes was more gradual, until a lowest value of 0.08 ± 0.01 × 10^9/L was reached, persisting for at least 4.5 hours. Only a partial recovery to 0.29 ± 0.11 × 10^9/L was observed after 24 hours (ie, immediately before administration of the second OKT3 dose).

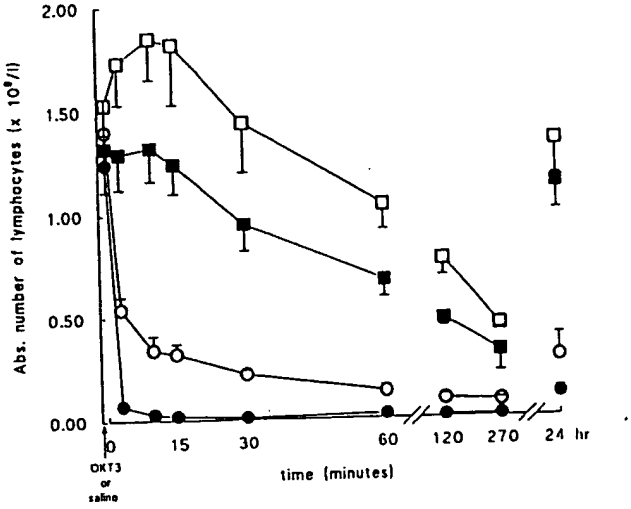

Fig 1. Absolute numbers of CD2⁺ and CD3⁺ lymphocytes on administration of 5 mg OKT3 or 500 mg methylprednisolone. Results are given as mean ± SEM in each group (n = 10). (○), number of CD2⁺ cells following OKT3; (●), number of CD3⁺ cells following OKT3; (□), number of CD2⁺ cells following methylprednisolone; (■), number of CD3⁺ cells following methylprednisolone.

Effect of OKT3 administration on the expression of adhesion molecules on peripheral blood lymphocytes. Data obtained before and at 3, 10, 60, 120 minutes, and 24 hours after administration of the first dose of OKT3 are summarized in Table 1. The MFI of CD62L on the remaining CD3 positive cells decreased within 120 minutes to 52.41 ± 12.86% of pretreatment value (P < .05). The MFI of CD11a on the remaining CD2 positive cells showed a 2.5-fold increase after 3 minutes and remained high for the whole study period, while the MFI of CD11a on the remaining CD3 positive cells increased much less. The MFI of NKI-L16 on the remaining CD3 positive cells showed hardly any change (Table 1). Before OKT3 administration, 22.86 ± 4.39% of the CD2 positive and 18.55 ± 3.71% of the CD3 positive lymphocytes were CD11b positive. However, from 10 minutes after OKT3 administration, almost all the remaining CD2⁺CD3⁻ cells were CD11b positive, and these CD11b⁺CD2⁺ cells followed the total number of CD2 positive lymphocytes. These remaining CD11b⁺CD2⁺CD3⁻ appeared to be also CD16 positive (data not shown), a phenotype characteristic for natural killer cells. The MFI of CD11b on the remaining CD2 positive cells showed a 1.8-fold increase within 3 minutes following administration of OKT3 and remained high for the whole study period. The MFI of CD11b on the remaining CD3 positive cells also showed a 1.9-fold increase within 10 minutes and remained elevated for at least 4.5 hours. No changes were observed in the percentage of CD49d positive cells or in the MFI of CD49d on the remaining CD2 positive and CD3 positive population after administration of OKT3 (data not shown).

In the 10 patients from the control group (treated with 500 mg methylprednisolone only), the absolute numbers of CD2⁺ peripheral blood lymphocytes initially increased from

Table 1. Expression of Adhesion Molecules on CD3 Positive and CD2 Positive Cells After Administration of 5 mg OKT3

Time (min)		CD3⁺	CD62L⁺CD3⁺	CD11a⁺CD3⁺	(NKI-L16)⁺CD3⁺	CD11b⁺CD3⁺
0	abs. no.	1.24 ± 0.15	1.06 ± 0.19*	1.30 ± 0.22	0.40 ± 0.07	0.23 ± 0.05
	MFI		100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3	abs. no.	0.07 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.04 ± 0.004
	MFI		89.49 ± 17.77†	137.48 ± 6.28	108.01 ± 2.92	168.60 ± 12.35
10	abs. no.	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.02 ± 0.003
	MFI		86.15 ± 17.70	145.33 ± 10.71	111.55 ± 5.04	187.27 ± 18.87
60	abs. no.	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.002	0.01 ± 0.01
	MFI		76.26 ± 11.87	133.30 ± 10.89	116.11 ± 9.75	227.01 ± 33.72
120	abs. no.	0.01 ± 0.004	0.01 ± 0.001	0.01 ± 0.004	0.01 ± 0.003	0.01 ± 0.004
	MFI		52.41 ± 12.86	121.81 ± 13.90	122.30 ± 7.05	196.33 ± 24.26
24 h	abs. no.	0.11 ± 0.03	0.07 ± 0.06	0.16 ± 0.09	0.01 ± 0.002	0.03 ± 0.01
	MFI		ND	ND	ND	ND
Time (min)		CD2⁺	CD62L⁺CD2⁺	CD11a⁺CD2⁺	(NKI-L16)⁺CD2⁺	CD11b⁺CD2⁺
0	abs. no.	1.40 ± 0.16	1.06 ± 0.19	1.40 ± 0.16	0.47 ± 0.05	0.32 ± 0.03
	MFI		100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3	abs. no.	0.54 ± 0.06	0.20 ± 0.04	0.55 ± 0.06	0.52 ± 0.06	0.48 ± 0.05
	MFI		74.53 ± 4.98	241.76 ± 20.21	125.75 ± 11.75	179.70 ± 18.62
10	abs. no.	0.34 ± 0.07	0.11 ± 0.03	0.34 ± 0.07	0.30 ± 0.08	0.31 ± 0.07
	MFI		69.61 ± 3.98	252.68 ± 22.25	126.42 ± 15.80	169.37 ± 17.55
60	abs. no.	0.13 ± 0.03	0.06 ± 0.05	0.20 ± 0.08	0.20 ± 0.08	0.18 ± 0.08
	MFI		69.93 ± 7.39	255.57 ± 24.16	125.57 ± 16.70	174.02 ± 18.59
120	abs. no.	0.09 ± 0.02	0.01 ± 0.003	0.09 ± 0.02	0.11 ± 0.02	0.08 ± 0.02
	MFI		75.32 ± 13.08	218.26 ± 16.67	123.71 ± 16.88	157.55 ± 20.28
24 h	abs. no.	0.29 ± 0.11	0.15 ± 0.06	0.30 ± 0.11	0.16 ± 0.07	0.18 ± 0.07
	MFI		107.91 ± 16.52	176.44 ± 10.14	136.21 ± 27.14	155.15 ± 36.95

All values represent mean ± SEM (n = 10).
Abbreviation: abs. no., absolute number; ND, not determined.
* × 10⁹/L.
† Percent of pretreatment value.

1.53 ± 0.23 × 10⁹/L before drug administration to 1.85 ± 0.20 × 10⁹/L at 10 minutes thereafter. From 15 minutes onwards, the CD2⁺ cell counts showed a gradual decrease to 0.45 ± 0.02 × 10⁹/L after 4.5 hours. After 24 hours, they had almost completely recovered to 1.35 ± 0.17 × 10⁹/L (Fig 1). The absolute number of CD3 positive cells remained constant during the first 15 minutes and then decreased and recovered parallel with the CD2 positive cells (Fig 1). No changes in the expression of the adhesion molecules CD62L, CD11a/CD18, NKI-L16, and CD49d/CD29 were observed from 15 minutes onwards, neither on CD2 positive nor on CD3 positive cells (data not shown). However the absolute number of CD11b⁺CD2⁺ cells increased, from 0.51 ± 0.06 × 10⁹/L before drug administration to 1.02 ± 0.09 × 10⁹/L at 10 minutes after saline administration, while the absolute number of CD11b⁺CD3⁺ cells remained constant during this time interval. The MFI of CD11b on the remaining CD2 positive and CD3 positive cells showed hardly any change (data not shown).

In Vitro Studies

Effect of OKT3 on the expression of adhesion molecules on peripheral lymphocytes. Addition of 1 μg/mL OKT3 or IXA to heparinized blood from five healthy volunteers did not induce any change in the absolute numbers of CD2⁺ cells, CD3⁺ cells, and CD16⁺CD56⁺CD3⁻ (natural killer) cells and neither did the expression of the adhesion molecules CD62L, CD11a/CD18, and CD49d/CD29 on CD2 positive and CD3 positive lymphocytes change (data not shown). In contrast, the number of CD3 positive lymphocytes expressing the activation epitope NKI-L16 on the CD11a/CD18 complex, showed a twofold increase within 3 minutes after addition of 1 μg/mL OKT3 (Fig 2A), followed by a gradual decrease. Addition of 1 μg/mL IXA also induced a twofold increase in the number of CD3 positive lymphocytes expressing the NKI-L16 epitope on the CD11a/CD18 complex, although more slowly as compared with OKT3 (Fig 2B). No changes were detected in the absolute numbers of CD11a⁺CD3⁺ cells (Fig 2A and B), nor in MFI of NKI-L16 and CD11a, either after OKT3 or IXA (data not shown). In addition, an immediate increase in MFI of CD11b on CD3 positive lymphocytes was observed on incubation with either OKT3 or IXA, reaching a maximum at three times the preaddition value after 60 minutes incubation (Fig 3A and B). Both the increase in NKI-L16 and CD11b expression were dose dependent, reaching a maximal effect with 10 μg/mL for OKT3 and with 1 μg/mL for IXA. A total of 0.1 μg/mL OKT3 or IXA still induced an increased expression of both adhesion molecules, but less than that observed with 1 μg/mL of each monoclonal antibody, and with 0.01 μg/mL OKT3 or IXA, no changes in adhesion molecule expression were detectable (data not shown). No changes

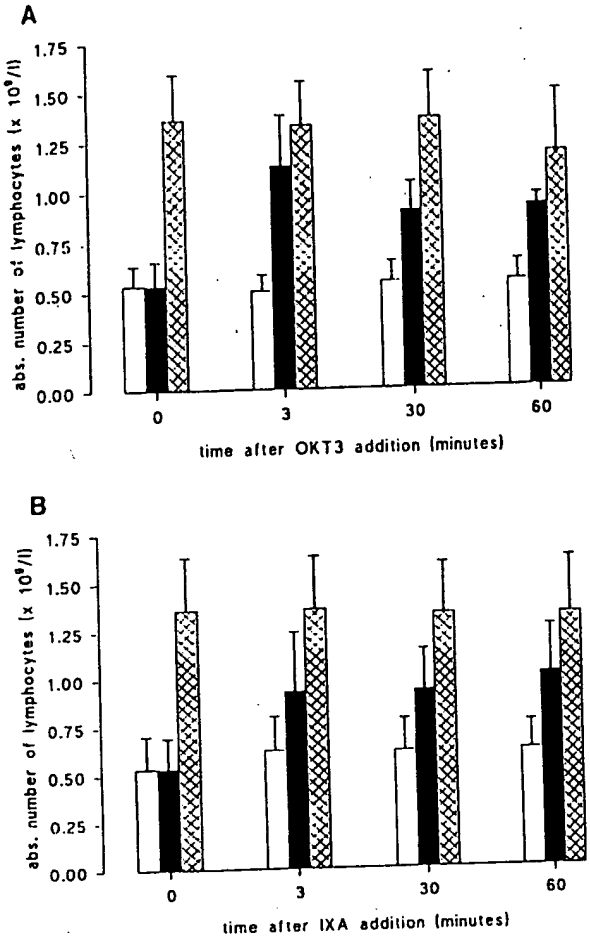

Fig 2. (A) Absolute numbers of [NKI-L16]$^+$CD3$^+$ and CD11a$^+$CD3$^+$ lymphocytes after addition of 1 μg/mL OKT3 or 1 μg/mL of an irrelevant murine IgG2a monoclonal antibody to whole blood samples of five healthy individuals. (B) Similar data after addition of 1 μg/mL IXA or 1 μg/mL of an irrelevant murine IgA monoclonal antibody. Results are given as mean ± SEM. Open bars, numbers of [NKI-L16]$^+$CD3$^+$ cells after irrelevant isotype control antibodies; solid bars, number of [NKI-L16]$^+$CD3$^+$ cells after OKT3 (A) or IXA (B); cross-hatched bars, number of CD11a$^+$CD3$^+$ cells after OKT3 (A) or IXA (B).

occurred in the expression of NKI-L16 or CD11b on CD3 positive cells in control samples incubated with murine IgG2a monoclonal antibodies directed against either the CD4 molecule or against a MHC class-I antigen, in any concentration tested (data not shown). Irrelevant murine monoclonal antibodies of the IgG2a or IgA isotype did not induce any alterations in expression of these adhesion molecules (data not shown).

Functional adhesion studies. Functional adhesion studies were performed to test whether the increase in expression of NKI-L16 and CD11b/CD18 after incubation with OKT3 or IXA affected the adhesion capacity of the T cells. Indeed, incubation of purified T cells with 1 μg/mL OKT3 or IXA in vitro caused a transient increase in adhesiveness of these cells to endothelial cell monolayers. Figure 4A demonstrates that after 3 minutes incubation of OKT3-coated T cells with goat antimouse Ig at 37°C, an adhesion score of 4.15 ± 0.22 was reached. Three minutes incubation of IXA-coated T cells with goat antimouse Ig at 37°C resulted in an adhesion score of 3.11 ± 0.26. The increased adhesiveness appeared to be transient, because after 30 or 60 minutes incubation, the adhesion scores decreased markedly (Fig 4A). Dose response curves showed that maximal adhesion of T cells to endothelium was observed with a concentration of 1 μg/mL of either OKT3 or IXA. A total of 0.1 μg/mL OKT3 or IXA could still induce T-cell adhesion to endothelium, but less than that observed with 1 μg/mL of each monoclonal antibody, while with 0.01 μg/mL OKT3 or IXA, no changes in adhesion could be detected (data not shown). As a positive con-

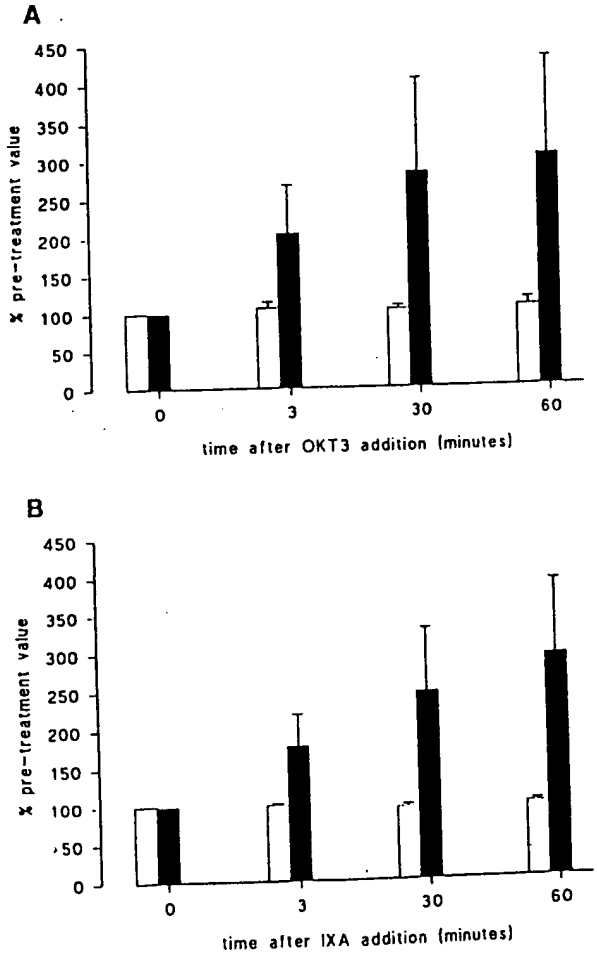

Fig 3. (A) MFI of CD11b on CD3$^+$ lymphocytes after addition of 1 μg/mL OKT3 (closed bars) or 1 μg/mL of an irrelevant murine IgG2a monoclonal antibody (open bars) to whole blood samples of five healthy control individuals. (B) Similar data after addition of 1 μg/mL IXA (closed bars) or 1 μg/mL of an irrelevant murine IgA monoclonal antibody (open bars). Results are represented as mean ± SEM.

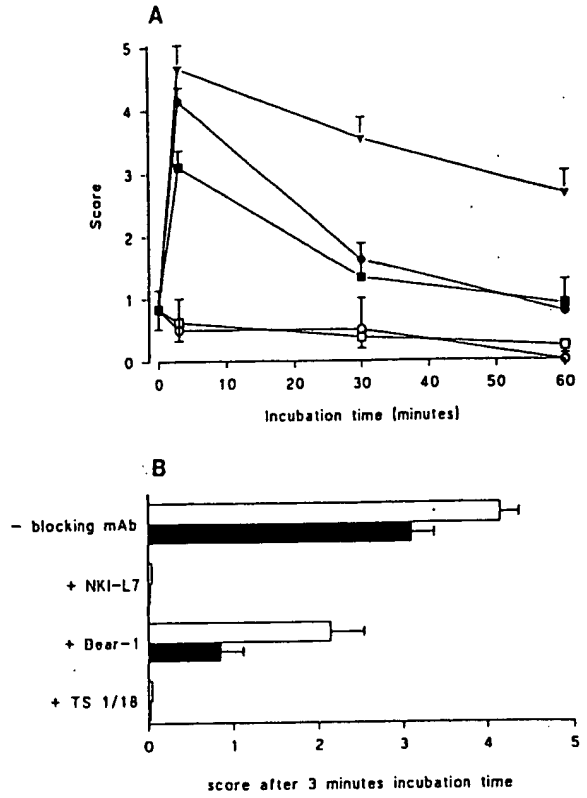

Fig 4. (A) Adhesion of purified T cells to monolayers of endothelial cells. Results are represented as mean ± SEM (n = 8). (●), 1 μg/mL OKT3; (■), 1 μg/mL IXA; (▼), 50 ng/mL phorbol myristate acetate, (□), 1 μg/mL of an irrelevant murine IgG2a monoclonal antibody; (○), 1 μg/mL of an irrelevant murine IgA monoclonal antibody. (B) Blocking of anti-CD3 monoclonal antibody-induced adhesion of purified T cells to endothelial cell monolayers with the monoclonal antibodies NKI-L7, Bear-1, and TS 1/18, directed against the α chain of the CD11a/CD18 complex, the α chain of the CD11b/CD18 molecule, and the common β, integrin (CD18), respectively. Results are given as mean ± SEM. Open bars, 1 μg/mL OKT3; solid bars, 1 μg/mL IXA. Adhesion score 0, no T cells adherent to endothelium; 1, < 50 T cells/field; 2, 50 to 100 T cells/field; 3, 100 to 250 T cells/field; 4, 250 to 350 T cells/field; 5, >350 T cells/field, scored independently at a magnification of 200 × by three technicians.

trol, T lymphocytes were incubated with 50 ng/mL phorbol myristate acetate. This resulted in an adhesion score of 4.67 ± 0.37. As a negative control, T cells were incubated with murine IgG2a monoclonal antibodies directed against either CD4 or a MHC class-I antigen. No changes in adhesion score were observed in any of the concentrations tested (not shown). Irrelevant murine monoclonal antibodies of the IgG2a or IgA isotype did not induce any alterations in adhesion score (Fig 4A).

The theoretical possibility that circulating human antimouse antibodies, which could be present in serum and can bind to the CD3 monoclonal antibody on one site and with Fc receptors on the other site, was excluded because the presence of human antimouse antibodies could not be detected by FACS analysis after incubating CD3 monoclonal antibody-coated cells with human autologous serum (data not shown).

Blocking studies showed that coincubation of OKT3- or IXA-coated T lymphocytes with either a CD18 or a CD11a monoclonal antibody completely inhibited the increased adhesion as observed after 3 minutes incubation with goat antimouse Ig (Fig 4B). Coincubation with a CD11b monoclonal antibody resulted in partial inhibition (Fig 4B).

DISCUSSION

In line with previous reports, the present study demonstrates that administration of the murine IgG2a antibody OKT3 causes an immediate peripheral T lymphocytopenia. Studies as to the causative mechanism(s) of this initial rapid disappearance of $CD2^+CD3^+$ lymphocytes are hampered by this very same phenomenon, because cells are no longer available for functional studies ex vivo. The decrease in the expression of CD62L on $CD3^+$ T lymphocytes may reflect activation, leading to shedding of CD62L from the cell surface.[20,21] Alternatively, it could reflect a preferential disappearance from the peripheral blood compartment of T cells with high CD62L expression. The latter possibility is supported by our observation that in the experiments in vitro, no change in expression of CD62L on T cells occurred. The increase in the percentage of $CD11b^+$ and $CD16^+$ cells within the remaining $CD2^+CD3^-$ population suggests that the observed increase in expression (MFI) of CD11a and CD11b and the decrease in MFI of CD62L on the remaining $CD2^+CD3^-$ lymphocytes results from a selective enrichment in the peripheral blood compartment of natural killer cells, which are indeed known to have a higher CD11a and CD11b expression,[22] (unpublished results), as well as a lower CD62L expression[21] as compared with T lymphocytes. The increase in MFI of CD11b on the remaining CD3 positive lymphocytes might reflect an actual upregulation of this adhesion molecule, as was already described by Muto et al.[22] These investigators showed that 30 minutes stimulation of T cells with 10 ng/mL phorbol myristate acetate at 37°C resulted in a twofold increase in the MFI of CD11b on T cells, as well as a twofold increase in the percentage of T cells expressing CD11b. This increased CD11b expression on T cells could not be blocked by cycloheximide, suggesting the presence of cytoplasmic (granule) stores of CD11b in the T cells. Phorbol myristate acetate is a direct activator of protein kinase C. Activation of T cells via the T-cell receptor/CD3 complex can also stimulate the inositol phospholipid mechanism, thereby giving rise to activation of protein kinase C,[12] which, in turn, may result in a rapid increase in CD11b expression.

To discriminate between the two possibilities of either redistribution of cells between peripheral blood and other lymphoid compartments or real upregulation of adhesion molecules, the expression of these adhesion molecules was also measured on peripheral blood lymphocytes in vitro at various time points after addition of OKT3, as well as IXA, to whole blood of healthy control individuals. If IgA antibodies can indeed cause an upregulation of adhesion molecules, this could explain the immediate peripheral blood lymphocytopenia that occurs after in vivo administration of a CD3

monoclonal antibody of the IgA class,[7] despite the absence of Fc receptors for murine IgA in humans and the inability of murine IgA to activate human complement.

In contrast to the results obtained from the studies with OKT3 in vivo, no change in expression of CD11a was observed in vitro, although an immediate, marked increase was observed in the absolute numbers of NKI-L16 positive T cells after addition of either OKT3 or IXA. Because the monoclonal antibody NKI-L16 recognizes a Ca^{2+}-dependent activation epitope on the CD11a/CD18 complex, its expression can be used as a parameter for an activation state of this adhesion molecule. However, it is known that expression of the NKI-L16 epitope by itself is not sufficient for cell-cell adhesion. Only after specific triggering of a lymphocyte by CD3 monoclonal antibodies through the T-cell receptor/CD3 complex, the NKI-L16 epitope becomes capable of high-affinity ligand binding.[12,13] Thus, the increased expression of this activation epitope, as demonstrated in the present study, may quite well play a role in the mechanism underlying the rapid decrease of $CD11a^+$[NKI-L16]$^+CD2^+CD3^+$ cells in the peripheral blood compartment after OKT administration, as these cells will strongly adhere to ICAM-1 and ICAM-2 on vascular endothelium. In contrast, $CD11a^+$[NKI-L16]$^+CD2^+CD3^-$ cells would remain in the circulation, not being activated via the T-cell receptor/CD3 complex, which is a prerequisite for induction of the high-avidity state of the CD11a/CD18 molecule. The observed increase in expression of CD11b on CD3-positive lymphocytes after the addition of either OKT3 or IXA in vitro is compatible with our findings in vivo after administration of 5 mg OKT3 and may play an additional role in adhesion of T lymphocytes to ICAM-1 on vascular endothelium.

Indeed, incubation of T lymphocytes with either OKT3 or IXA appeared to induce an increased, transient adhesiveness of these cells to endothelial cell monolayers within 3 minutes. Because this increase was completely inhibited in the presence of CD11a or CD18 monoclonal antibodies, and partly in the presence of a CD11b monoclonal antibody, the induced activation status of the CD11a/CD18 molecule appears to be an absolute prerequisite for the immediate adhesion of T cells to vascular endothelium as induced by CD3 monoclonal antibodies. However, part of the increased adhesion is also dependent on increased expression of CD11b/CD18.

The $CD2^+CD3^-$ lymphocytes that remained detectable in the peripheral blood compartment appeared to be $CD11b^+$ and $CD16^+$, a phenotype characteristic for natural killer cells. The ongoing decrease in absolute number of such natural killer cells between 60 and 120 minutes after the first OKT3 administration might be caused by a release of interleukin-2, which is known to occur within 2 hours after the first dose of 5 mg OKT3.[23,24] Interleukin-2 is able to induce an immediate selective disappearance of natural killer cells from the peripheral blood,[25] as can be explained by an increased adhesion of these cells to endothelium as was shown by in vitro studies.[25-27] In contrast, administration of methylprednisolone did not induce a disappearance, but a transient increase of $CD11b^+CD2^+$ cells, followed by a gradual, mild decrease, as described previously.[28,29]

The persistence of lymphocytopenia after administration of OKT3 (or other anti-CD3 monoclonal antibodies of the IgG2a isotype) may be caused by at least two possibly cooperating mechanisms: It may result from opsonization of T lymphocytes by OKT3 and complement, followed by sequestration of such sensitized lymphocytes in the mononuclear phagocyte system. Alternatively, it may result from upregulation of VCAM-1 and ICAM-1 on the endothelium, as induced by cytokines like tumor necrosis factor α and interferon γ, which are known to be released following OKT3 administration[23,24] and which may lead to increased adhesiveness of the endothelium for its counterstructures CD49d/CD29 and CD11a/CD18 or CD11b/CD18, respectively, expressed on peripheral blood lymphocytes.[30-33] Evidence for upregulation of VCAM-1 by CD3 monoclonal antibodies was provided by Bergese et al[34] after administration of the hamster monoclonal antibody 145-2C11 in mice.

Because the murine IgA isotype switch variant CD3 monoclonal antibody does not bind to human Fc receptors, does not induce complement activation, and hardly results in release of cytokines, administration in vivo of IgA anti-CD3 antibody does not lead to persistent lymphocytopenia.[8,9]

In conclusion, we provide evidence that on administration of CD3 monoclonal antibodies in vivo, the immediate peripheral blood lymphocytopenia is, at least partly, explained by changes in the expression of adhesion molecules on the surface of T lymphocytes, leading to increased adhesiveness of these cells to vascular endothelium.

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